Concepts for Injectable Nanoparticles for *In Vivo* Removal of Overdose Toxins from Blood

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- This paper represents an interdisciplinary effort recently started by a group of engineers, medical doctors and scientists at the University of Florida, an effort designed to mediate the loss of life by a large number of people who have become overdosed internally or externally with toxic chemicals.

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Vision

Develop cost-effective medically useful nanoparticles containing redundant engineered systems for:

1) Prevention of the toxic effects of chemical warfare agents (CWA) (prophylaxis to maintain combat readiness)

2) Effective treatment of CWA-intoxicated personnel

3) Effective decontamination of CWA-infected combat zones

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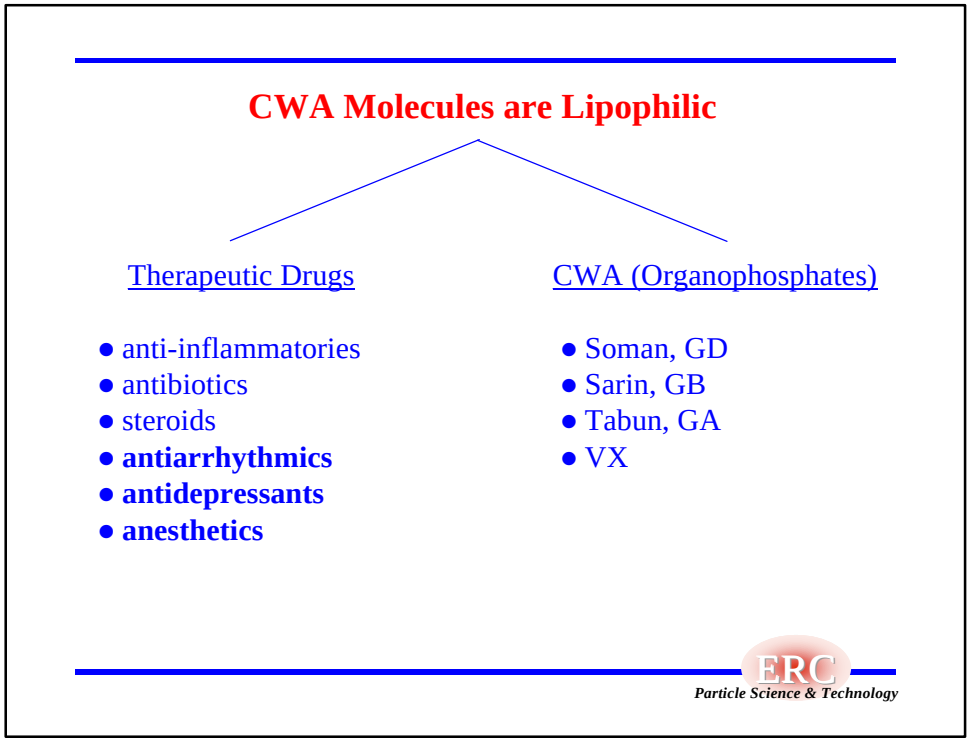
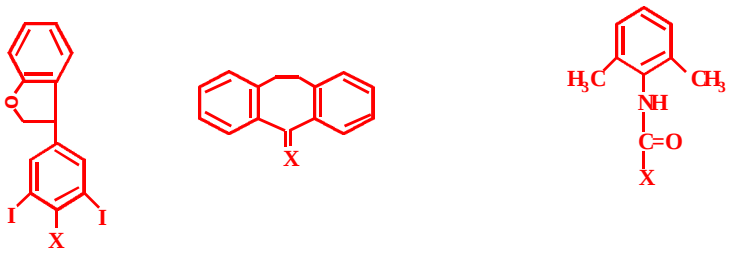
- The vision for the technology to be developed, controlled and selective toxic chemical removal, is that it will serve to fill the void that exists in emergency medical treatment for overdosed persons and contaminated environments. The technology will be equal in importance to but opposite in concept to controlled release delivery of pharmaceuticals.



- The toxic chemicals of concern include prescription therapeutics such as antiarrythmic, antidepressant and local anesthetic drugs, as well as terrorist and warfare agents. All are lipophilic.

Drugs currently being investigated

Amiodarone	Amitriptyline	Bupivacaine
Antiarrhythmic	Antidepressant	Anesthetic



where X = aminoalkyl (similar to VX)

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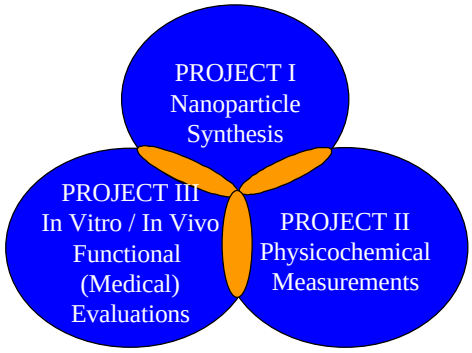
- Initially, the research will focus on creating nanoparticles to efficiently remove three therapeutic drugs which cause thousands of deaths each year. All are aromatic and basic due to an aminoalkyl group pendant to a benzene ring. It will be demonstrated that the pi electron density in at least bupivacaine is high enough to allow its selective binding to a pi acceptor aromatic, hence a means for detoxification. Chemical warfare agents are typically nonaromatic so their removal will rely on their lipophilicity and partitioning from blood onto hydrophobic particles or into oil-in-water microemulsions.

Components of Proposed CWA Research Program



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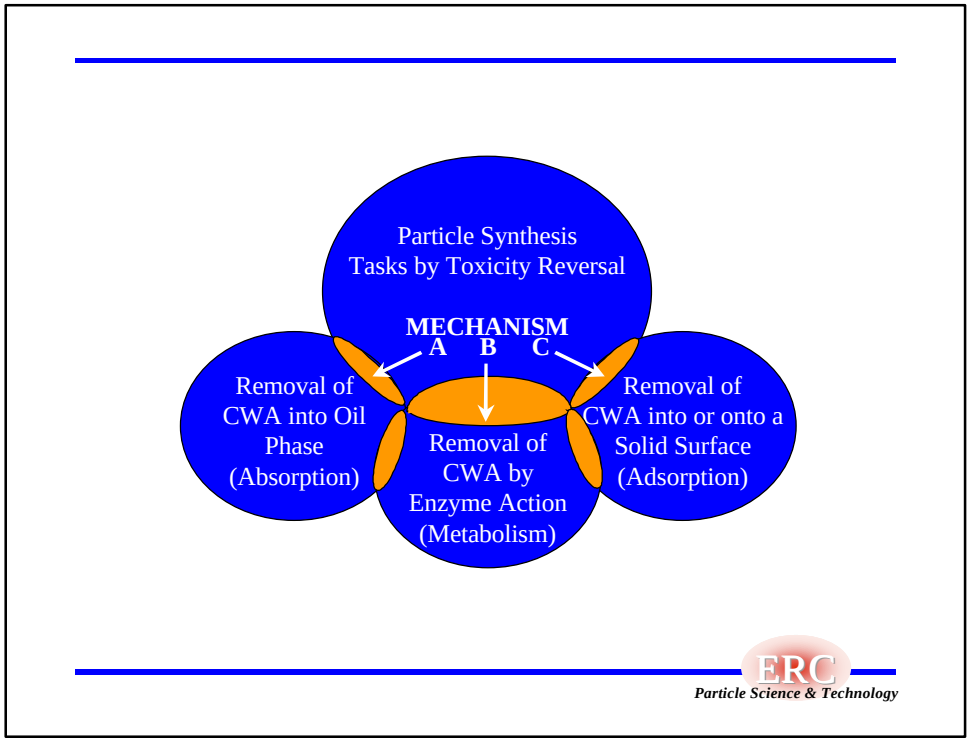
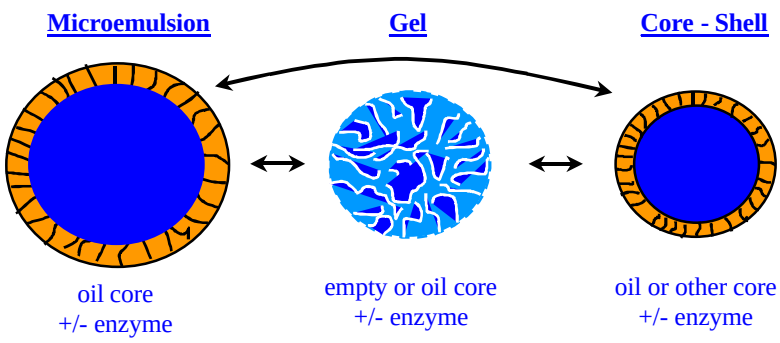
- The overall goal will be achieved through three areas of investigation: nanoparticle synthesis; physicochemical characterization and evaluation of the types of nanoparticles generated; and in vitro and in vivo testing of toxic chemical removal capabilities.



- Removal of overdose toxins from the blood stream is considered to be possible using one of three mechanisms: (A) partitioning or absorbing the generally lipophilic toxins into nano and micro emulsion oil droplet reservoirs; (B) degrading or metabolizing the toxins using natural or more active expressed enzymes; and (C) adsorbing the toxins onto solid nanoparticle surfaces. These mechanisms might be employed singly or in some combination.

Particle Synthesis for CWA Removal Mechanisms A/B



Enzyme = CWA Degradation (e.g., human paraoxonase)

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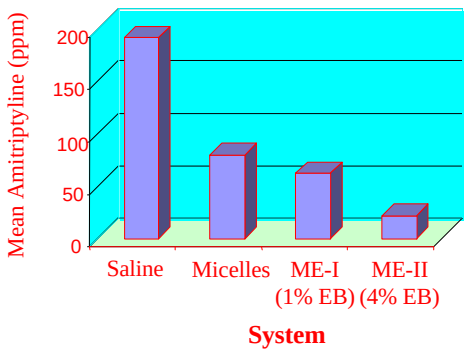
- Particles envisioned for use by removal mechanisms (A) and (A)/(B) are microemulsions where the oil core is stabilized by one or more of several biocompatable surfactants. Alternatively, the oil-like cores to be evaluated may be in gel form. The oil or gel cores may be encapsulated in a rigid shell, the latter of which may be prepared with molecularly templated pores to allow selective passage of one or other of the small toxins but not macromolecules contained in blood. Finally, the enzyme if included may be natural or expressed and be selected for a given target toxin.

Effect of pluronic L-44 micelles and microemulsions (ME) to reduce the concentration of amitriptyline in human plasma



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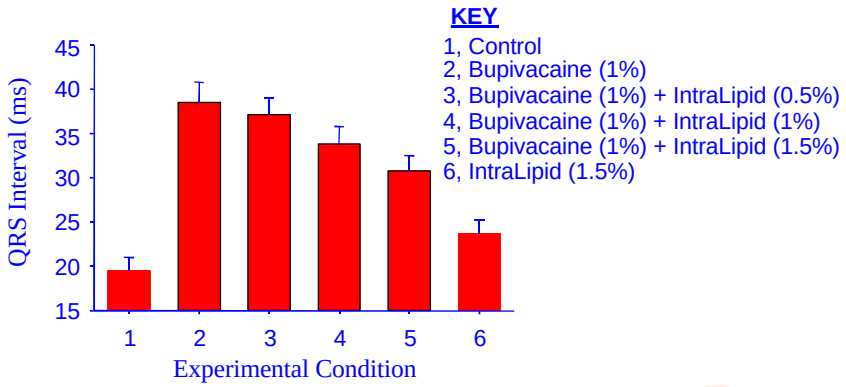
- Proof of concept that oil-in-water microemulsion droplets are effective in removing the antidepressant drug from blood plasma has been established (Shah). The efficiency of removal depends on the way the microemulsion is prepared. The oil in this study was ethyl butyrate.

Attenuation of The Cardiotoxic Effects of Bupivacaine in Guinea Pig Isolated Heart by Macroemulsion

In Vitro-Whole Heart (Tissue) Level



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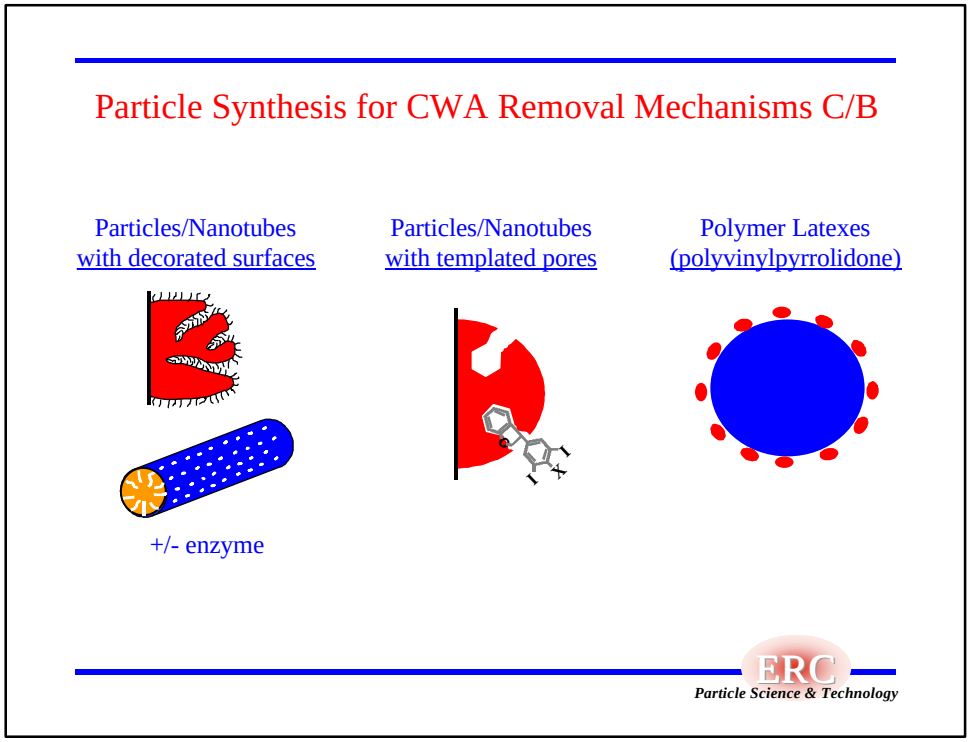
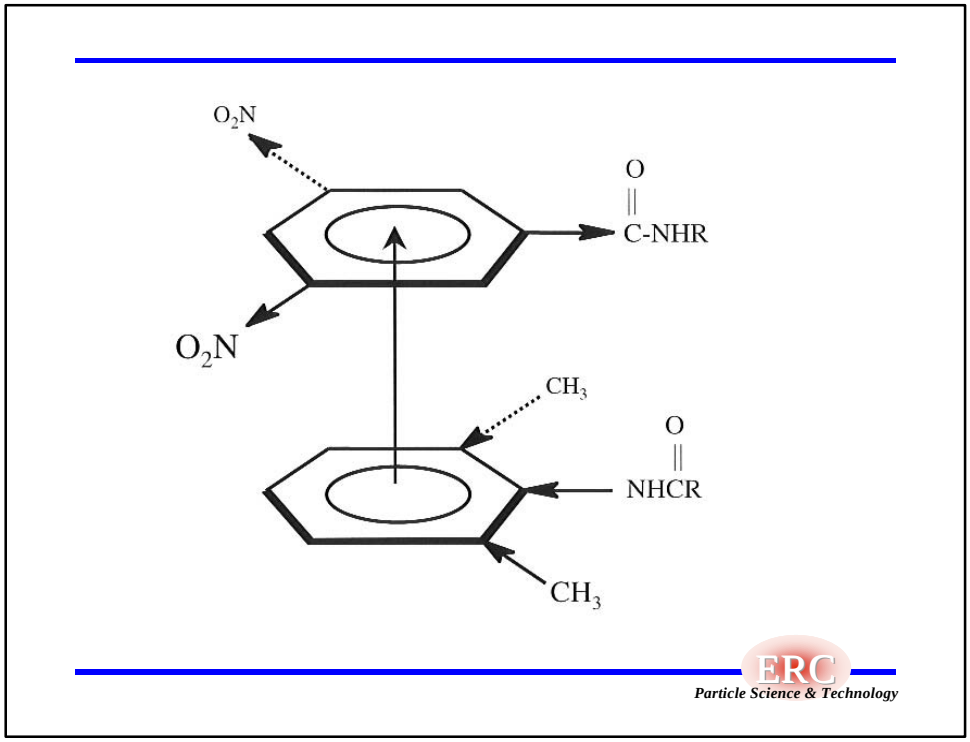
- In vitro tests on reduction in concentration of the toxic anesthetic bupivacaine in heart muscle by a natural oil-in-water emulsion have been successful (Dennis and Morey).



- Nanoparticles envisioned for use by removal mechanisms (C) and (C)/(B) are solids having different shapes, porosities and decorated surfaces. Particles and nanotubes may have hydrophobic molecules attached capable of acting as oil-like sites into which the lipophilic toxins will migrate. The attached molecules may have additional features for selective binding with toxins. The nanotubes may have such attachments either or both on the inside or outside. Solid nanoparticles will be prepared with pores templated for the specific toxin to be removed. Templating of inorganic metal oxides and of organic polymers has precedent and will be applied to the nanoparticles of interest in this research (Partch). In the center example one hexagonal end of amiodarone is shown fitting into a templated pore. Finally, solid latex cores composed of polymer known for drug delivery will be adapted for use. PVP is one such polymer which, due to its water solubility, will be encapsulated or otherwise altered for toxin removal studies (Partch).



- Benzene rings having opposite degrees of pi electron electron density form complexes with each other. Here the upper ring substituted with -NO2 and -C=O groups is electron deficient. It attracts (vertical arrow) pi electron density from the lower ring substituted with -CH3 and -NH groups. It should be noted that the lower ring structure is identical to that in the toxin bupivacaine where R = aminoalkyl. The upper acceptor ring is designed for easy covalent attachment to a silica nanoparticle (R= -(CH2)n-SiO2 .

Values of Δ for Donor-N-Methyl-3,5-dinitrobenzamide Complexes[a] in Chloroform-d			
Donor	**Resonance**	**Δ (ppm, acceptor)**	**Direction**
2,6-Dimethylaniline	Triplet	0.0874	Upfield
	Doublet	0.0779	Upfield
2,6-Dimethylacetanilide	Triplet	0.0156[b], 0.1584	Upfield
	Doublet	0.00775[b], 0.0055	Downfield, Upfield
Bupivacaine (salt)[c]	Triplet	0.0891	Upfield
	Doublet	0.0275	Upfield
[a] Donor : Acceptor = 60 : 1, except case b where D : A = 1 : 1; [c] Studied in 50 : 50 D_2O : CD_3CN			

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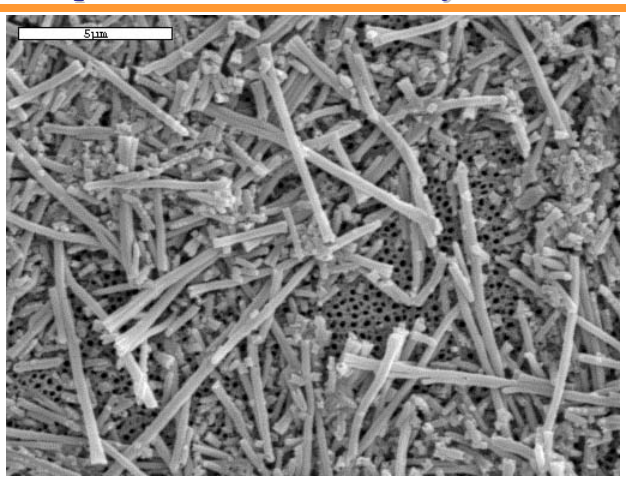
- Proof of concept has been demonstrated that the pi-donor pi-acceptor molecules selected form a complex (Partch). The table shows previously unknown data for the change (delta) in NMR proton chemical shift values of the doublet and triplet signals for the pi-acceptor dinitrobenzamide derivative. The upfield direction and magnitude of the changes compares with literature values when trinitrobenzene was the pi-acceptor for various aralkyl compounds.

Scanning Electron Micrograph of the SiO_2 Nanotubules After Dispersion and Collection by Filtration



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- Preliminary results on use of 30 nm diameter silica nanotubes decorated with either C18 chains or with an enzyme on their interiors are encouraging (Martin). Shown are the tubes before undergoing internal surface modification. After treatment the tubes showed effective removal of a tricyclic aromatic from solution, and glucose oxidation by tethered oxidase enzyme.

Project II - Physicochemical Characterization of Nanoparticle-CWA Interaction

Applicable Analytical Techniques

- Spectroscopy
- Chromatography
- Liquid Scintillation
- Ultrafiltration

Phenomena to be Observed

- Kinetics of CWA capture
- Nanoparticle-CWA interactions with blood components
- Equilibrium distribution of CWA between solutions and nanoparticles

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- During and after the nanoparticle synthesis, all microemulsions, gels and decorated particles will be fully characterized as to composition, morphology and affinity for removing various toxins from synthetic solutions and blood plasma.

Project III - *In Vitro* and *In Vivo* Functional Assessment of Nanoparticles

Biological Tests for NSF Detoxification Program (drugs = amiodarone, amitriptyline, bupivacaine, i.e., 1^{o} toxicity is heart-related)

***In Vitro* Studies**

- Isolated hearts
  - --His bundle electrograms (heart conduction)
  - --Monophasic action potentials (heart repolarization)
- Isolated heart cells (ventricle)
  - --Currents (e.g., Na current)

***In Vivo* Studies (Whole animal)**

- EKG
- Arterial pressure

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- The biomedical efficacy of the types of nanoparticles under preparation for removal of the three toxic therapeutic drugs will be evaluated both in vitro and in vivo (Dennis, Morey).

Project III - *In Vitro* and *In Vivo* Functional Assessment of Nanoparticles for CWA Detoxification

Biological Tests for Proposed CWA-nanoparticle program

- modified approach necessary

Assessment of CWA toxicity in 3 major organ systems:

- Central Nervous System – EEG (Seizure activity)
- Cardiovascular System – EKG (Arrhythmias)
- Respiratory System – Ventilatory status (Paralysis)

Other Measures of Nanoparticle Efficacy

- Survival and LD_{50} estimates

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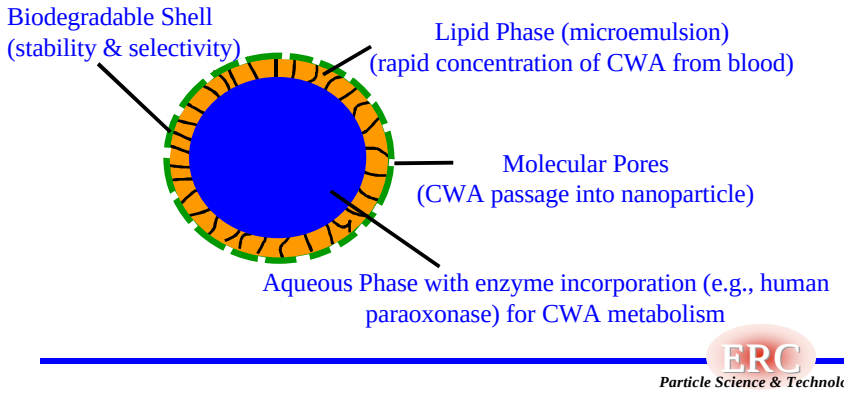
- The ability of the nanoparticle systems to remove warfare and terrorist chemicals requires slightly different in vitro and in vivo medical protocols than the assessments for the therapeutics (Dennis, Morey). For the CWA toxins CNS and respiratory activity will be monitored using appropriate organs.

What is the potential of nanoparticles to safely and effectively deal with CWAs?

2 illustrative examples of what nanoparticles can offer:

1. Substrate Concentration Effect for CWAs



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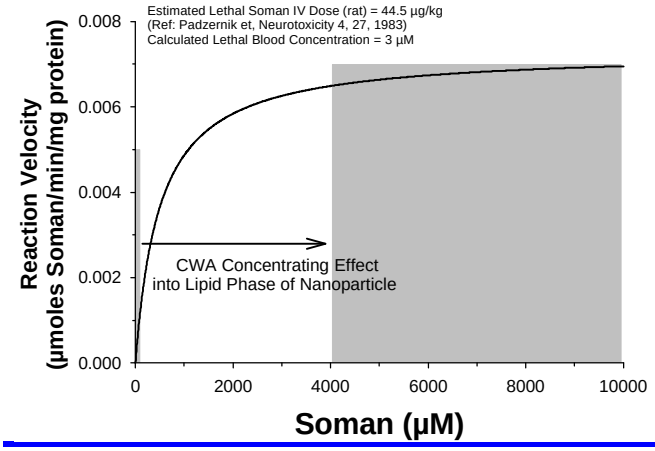
- Two examples are envisioned for how the selected nanoparticles might function to reduce overdose toxins. The first is illustrated here where the idealized nanoparticle is composed of several core-shell compartments. Note this example employs a water-in-oil core surrounded by functional shells.

Substrate Concentration Effect for CWA

Enzyme = Phosphotriesterase
K_M = 500 µM
V_{Max} = 0.0073 µmoles/mg/min

Reference Dumas *et al* Arch Biochem Biophys 277:155, 1990.



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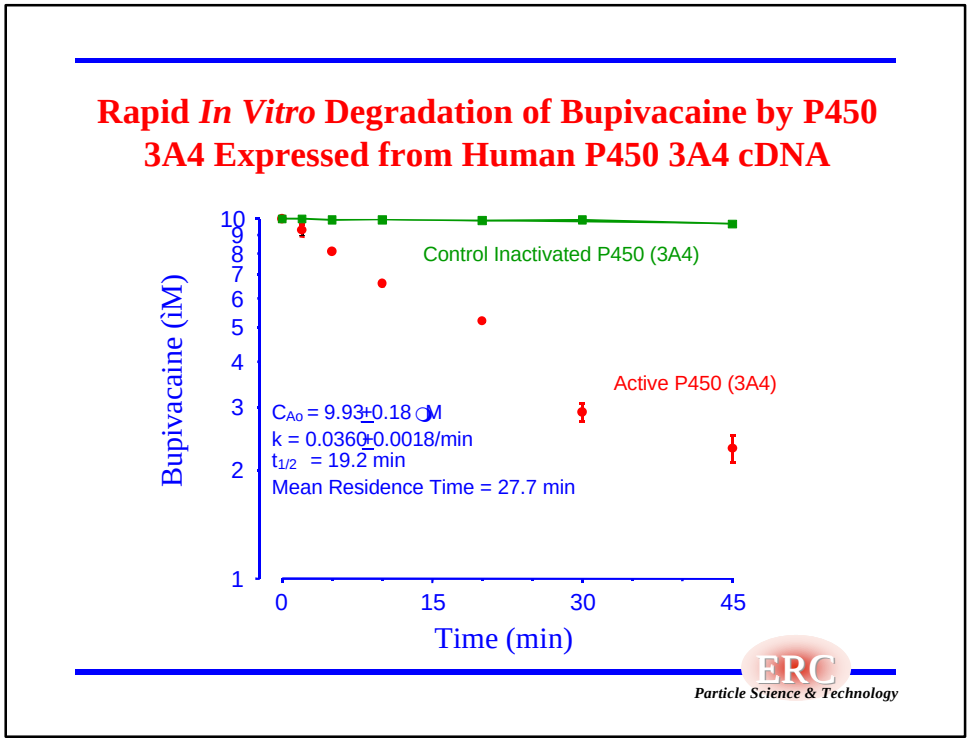
- The design of the previously shown and described idealized particle is such that it is intended to be a reservoir in which the toxin will be concentrated over 1000X above the lethal dose (3 uM for Saran) and thereby facilitate the rate of enzyme attack and toxin destruction.



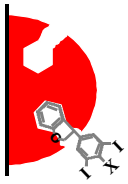
- Representative of enhanced activity by expressed enzyme are the rates of degradation of bupivacaine toxin shown in the figure. The half-life is much reduced from its value when natural P450 is used.

What is the potential of nanoparticles to safely and effectively deal with CWAs?

2. Adsorption of CWAs onto Nanoparticles ("nanobodies")

Particles/Nanotubes with decorated surfaces

Particles/Nanotubes with templated pores



- Similar to antibodies in their function (e.g., digibind)
- Potential to bind CWAs and excrete them from body via kidneys (nanoparticles ⊙5 nm diameter)

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- The second example of how the nanoparticle may function to detoxify blood is to employ adsorption using solids having either decorated surfaces or molecularly templated pores.

Conclusions

Nanoparticle technology offers unprecedented cost-effective opportunities to maintain combat readiness in CWA zones by providing:

- A therapeutic approach to safely and efficaciously prevent (and treat) CWA toxicity

- An approach to safely, rapidly and effectively cleanse CWA-contaminated combat zones